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High resolution characterisation of *E. coli* proliferation profiles in livestock faeces

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ABSTRACT

Agricultural intensification can lead to high volumes of livestock faeces being applied to land, either as solid or liquid manures or via direct defecation, and can result in reservoirs of faecal indicator organisms (FIOs) persisting within farmland. Understanding the survival of FIOs, e.g. *E. coli*, in agricultural environments, and in particular within different livestock faeces, is key to developing catchment management practices for the protection of ecosystem services provided by clean water. Frequently, controlled laboratory studies, under constant temperature regimes, are used to determine the impact of environmental factors on *E. coli* persistence in livestock faeces; however, such studies oversimplify the diurnal variations and interactions of real world conditions. The aim of this study was to investigate the survival of *E. coli* using a controlled environment facility, which simulated diurnal variation of temperatures typically experienced during a British spring and summer. The approach provided a comparison of *E. coli* persistence profiles within faeces of sheep, beef cattle and dairy cattle to allow novel interpretations of *E. coli* regrowth patterns in contrasting livestock faeces in the period immediately post-defecation. Thus, the coupling of a tightly controlled environment facility with high resolution monitoring enabled the development of a new non-linear, asymptotic description of *E. coli* proliferation in livestock faeces, with increased potential for *E. coli* growth observed during warmer temperatures for all livestock types. While this study focused on temperatures typical of the UK, the occurrence of a phase of *E. coli* regrowth has implications for microbial water quality management worldwide.

1. Introduction

Increased demand for food production has led to approaches that aim to deliver sustainable intensification in agricultural systems (Rockström et al., 2017). Despite best efforts to promote sustainable intensification, the need to feed a growing population can still lead to poor management of livestock, and the unsustainable use of agricultural land and (in)organic fertilisers, with the potential to impact negatively on wider environmental quality (Yang et al., 2016). For example, increased livestock numbers on-farm could lead to higher volumes of livestock faeces being applied to land, either as manure, slurry or via direct defecation, introducing large quantities of faecal indicator organisms (FIOs) to agricultural landscapes. Importantly, the mobilisation and delivery of FIOs to receiving waters following rainfall threatens important ecosystem services related to clean and safe drinking, bathing and shellfish harvesting water (Clements et al., 2015; Kay et al., 2018; Pandey et al., 2018).

E. coli is the most routinely monitored FIO in environmental samples, though its detection does not imply the presence of pathogenic microorganisms in the same sample (Bradshaw et al., 2016; Pachepsky et al., 2016). However, detection of *E. coli* in soil or water does indicate faecal contamination of the environment. The magnitude of *E. coli* burden contributed to land from agriculture is therefore a useful index when assessing the vulnerability of nearby watercourses to microbial pollution risk (Dymond et al., 2016). Understanding how the landscape burden of *E. coli* varies in space and time is challenging, due to the complex survival dynamics of *E. coli* under different abiotic conditions (Dusek et al., 2018). A particularly important source of *E. coli* in agricultural landscapes is freshly excreted livestock faeces which, unlike most slurry and farmyard manure, does not undergo any storage or treatment prior to land application and therefore often contains a higher concentration of FIOs (Vinten et al., 2004).

Controlled laboratory studies, under constant temperature regimes, have been used extensively to determine the impact of

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specific environmental factors on *E. coli* persistence in livestock faeces. Outputs from such studies have been deterministic first-order decay functions that describe the exponential die-off of the target population under different temperatures (Wang et al., 2004), dry matter content of protective media (Ishii et al., 2010) or contrasting soil types (Lau and Ingham, 2001). To complement the mechanistic understanding delivered via controlled laboratory studies, field-relevant investigations have profiled *E. coli* persistence in livestock faeces exposed to combinations of variable and interacting environmental factors (e.g. Oliver and Page, 2016; Moriarty et al., 2011; van Kessel et al., 2007). This field-relevant research has identified significant deviations from the first-order decay functions observed under controlled conditions, with *E. coli* cell growth and protracted survival leading to much longer persistence than that predicted from first-order die-off models (Brouwer et al., 2017).

Whether research has opted for a field or laboratory focus, there has been little direct comparison of persistence profiles in multiple faecal types under contrasting conditions. Most research has focussed on bovine faeces (Ashekuzzaman et al., 2018; Martinez et al., 2013) with relatively little information currently available for ovine faeces (Moriarty et al., 2011; Hodgson et al., 2009). Furthermore, the observed growth phase of *E. coli,* commonly identified in field-relevant studies, represents an interesting shift in our understanding of *E. coli* survival; however, knowledge of what governs the rate and magnitude of post-defecation *E. coli* cell growth is lacking (Oliver et al., 2016). This problem is compounded by observations of *E. coli* growth occurring at the beginning of long-term studies, leading to inferences of *E. coli* growth being based on only a few data points. Therefore, the modelling of *E. coli* persistence in faecal deposits justifies a piecewise approach whereby the initial growth phase is described separately from the subsequent decay phase. For the initial growth phase, non-linear modelling approaches may provide a better approximation of the system than the linear approaches employed previously (Oliver et al., 2010). For example, we might expect regrowth to be most rapid in fresh faeces, reducing through time as conditions within the deposit become less favourable. Thus, fitting an asymptotic model to describe the *E. coli* population growth rate, which declines as it moves toward a maximum, could provide an opportunity to improve upon linear modelling approaches.

While field-relevant studies are useful for investigating *E. coli* behaviour in the landscape, the complex mix of interacting environmental factors makes it difficult to identify the dominant drivers that govern *E. coli* persistence and growth in livestock faeces. Yet controlled static-temperature laboratory studies oversimplify real world conditions and rarely, if ever, capture growth as observed in the field (Park et al., 2016). The use of a more advanced controlled environment facility (CEF) offers the potential to minimise uncertainty from variable interacting factors but elevate the quality of simulated controlled conditions, e.g. by allowing diurnal temperature regimes and varying daylight hours, but have yet to be exploited for exploring *E. coli* persistence in the context of environmental management. The aim of this study was to investigate *E. coli* persistence in beef cattle, dairy cattle and sheep faeces, using a CEF. Our objectives were to: (i) use high-resolution sampling to quantify and model the potential for *E. coli* regrowth previously unaccounted for by controlled laboratory studies; (ii) determine whether *E. coli* regrowth profiles vary in different livestock faeces across contrasting seasonally-defined conditions; (iii) investigate the temperature sensitivity of *E. coli* persistence within different faecal types; and (iv) characterise differences in the *E. coli* hazard associated with faecal deposits from different livestock types.

2. Materials and method

2.1. Experimental climate chambers

All experiments were carried out in climate cabinets, which were designed to allow multifactorial climate manipulation (Snijders Microclima 1750E, Tilburg, Netherlands). Cabinets were set up to mimic diurnal temperature variation experienced during a typical British spring or summer, which represent key seasons for livestock grazing in the UK (cattle are typically housed over the winter). Two temperature treatments were used: (i) typical seasonal temperatures for spring and summer based on long term average datasets; and (ii) scenarios to test climate sensitivities for spring and summer. Temperature settings were derived from 30 year averages available from the Met Office MIDAS dataset (Met Office, 2012). For the climate sensitivity experiment, temperatures of 2 °C more than the MIDAS seasonal averages were used, based on UKCP09 projections. These data were acquired from the Scottish Climate Projections App (2017) with the Eastern Scotland region selected. The probability level used was 50% representing an equal chance of UKCP09 climate model realisations resulting in a temperature either above or below the temperature specified. Temperature in the CEF varied from an average minimum and maximum following a sinusoidal wave mimicking diurnal variation of temperature (Table 1). In order to simulate solar irradiance, timers were set to mimic periods of daylight and night time with UV strengths typical for the UK during the seasons of interest. UV activation periods were centred over the time of maximum temperature. Monthly means of solar irradiance were acquired from the SoDa Service (2013) and converted to a seasonal mean (Table 1).

2.2. Experimental design

To ensure that faeces used in the experiments was representative of the livestock diet typical for the season of interest, fresh faeces were collected during the respective season, and then transferred to the CEF. Faeces was collected from farms in Stirlingshire, Scotland and from the same herd/flock for the spring and summer treatments. For each temperature treatment, five in-tact replicates of dairy and beef cattle faeces, which were less than 12 h old, were collected. Dairy cattle faeces were collected from an area where cows were held prior to milking, which was cleaned twice a day. Beef cattle and sheep were grazing on pasture and the freshness of faeces was ensured by collecting deposits from the area immediately surrounding livestock. In order to collect enough faeces for the sheep experiment, each replicate was made up of

Table 1
Controlled environment facility settings.

Season	Minimum temperature (°C)	Maximum temperature (°C)	Temperature variation (°C)	Hours of daylight	UV (J/cm^2/day)
Spring	3.97	11.86	7.89	13.0	34.41
Spring +2 °C	5.97	13.86	7.89	13.0	34.41
Summer	10.2	18.34	8.14	15.5	36.30
Summer +2 °C	12.2	20.34	8.14	15.5	36.30

pellets from five fresh deposits which were collected and homogenised. Faecal deposits had an average fresh weight of 1516 g (sd = 350 g) and 1766 g (sd = 633 g) for dairy and beef cattle, respectively. The average fresh weight for groups of five faecal deposits from sheep was 116 g (sd = 58 g). All faeces were transferred into the climate cabinets on the day of collection. Every two days the faecal deposits were misted with sterile distilled water at a rate of 1 mL/100 cm^2 to mimic a 'morning dew' effect, and avoid a complete dehydration of the faeces under CEF conditions. Bovine faecal samples were collected for microbial analysis on a daily basis, and every other day for sheep faeces (as dictated by the smaller faecal volume associated with ovine faeces). Sampling was undertaken over a period of 20 to 30 days, depending on the volume of source material available. A small sample of faeces representing a cross section of the deposit, approximately 0.5 cm in diameter, was retrieved using a sterile spatula. This was carried out daily for 15 to 20 days after which the sampling frequency was decreased in order to retain enough material to lengthen the complete duration of the experiment to 30 days. Sampling ceased when further samples could not be taken without intersecting with areas previously sampled. The spatula used to sample the faeces was sterilised between replicates, and the faeces transferred into a sterile sample pot. Repeated sampling was used over destructive sampling; sampling repeatedly from the same faecal deposit assumed homogenisation of the generic *E. coli* population within the faecal matrix during passage through the livestock gut, as previously demonstrated by repeated spatial sampling of faecal material (Oliver, 2014).

2.3. Sample analysis

On each sampling occasion approximately 2 g of faeces was removed from each deposit; 1 g was used to determine moisture content by oven drying the sample at 105 °C for 24 h, with the remainder used for quantifying the concentration of *E. coli,* reported as colony forming units (CFU) g^{-1} dry weight faeces. The number of viable *E. coli* cells in faeces was determined using standard culture-based methods, and carried out within 30 min of the faecal samples being collected. Briefly, approximately 1 g of faeces was added to 9 mL of phosphate buffer saline (PBS) prior to shaking at 130 rpm for 30 min. The resulting slurry mix was then vortex mixed and serially diluted prior to inoculation onto membrane lactose glucuronide agar (MLGA) (CM1031, Oxoid; Basingstoke, UK) using the spread plate method. Agar plates were inverted and incubated for 24 h at 37 °C. All colonies counted represented presumptive *E. coli* and all sample analysis was performed in duplicate. Membrane filtration of samples was also used where necessary to complement the spread plate method and improve the limit of detection. Briefly, 1 mL of each serially diluted sample was mixed with approximately 20 mL of sterile PBS and filtered through sterile cellulose acetate membranes of 0.45 μm pore size (Sartorius Stedim Biotech; Goettingen, Germany) using a vacuum filtration unit (Sartorius). Membrane filters were then aseptically transferred to plates containing MLGA, inverted and incubated for 24 h at 37 °C. The limit of detection was 50 cells/g of wet faeces. Method blanks of PBS were used to ensure no contamination occurred during sample processing.

2.4. Statistical analysis

All *E. coli* counts underwent $\log_{10}$ transformation prior to statistical analysis. Distributions of *E. coli* were not log normally distributed as determined using the Anderson – Darling normality test and this was accounted for in subsequent data analysis.

We hypothesised that *E. coli* population growth within faecal deposits would likely be most rapid immediately following deposition, slowing as conditions within the deposit become less favourable for *E. coli* population growth. As linear modelling approaches assume a constant growth rate they would not be appropriate here; and whilst quadratic terms within a linear model can be used to address this problem, this approach can lead to predictions with negative values. Therefore, the use of more complex non-linear models was justified (Paine et al., 2012). The asymptotic exponential model provides an opportunity to investigate the magnitude and duration of *E. coli* growth. The asymptotic exponential form (Eq. (1)) predicts growth rate to be fastest initially, slowing to a stationary maximum and has three parameters: an intercept (initial *E. coli* concentration); a horizontal asymptote (maximum *E. coli* concentration); and a rate constant (speed of *E. coli* population growth).

$$y = a - be^{cx} \tag{1}$$

where a is the horizontal asymptote (Log_{10} cfu g^{-1}), b is the magnitude of growth (Log_{10} cfu g^{-1}) and c is the exponential rate constant (day^{-1})

Repeated measurements of *E. coli* concentration from a given faecal deposit are not independent and are likely to be serially related. Therefore, a mixed effects approach, which incorporates a random effect allowing a model to vary between individual deposits and a temporal dependence structure between measurements, was required (Pinheiro and Bates, 1995). Three temporal autocorrelation structures were tested: auto regressive order 1; compound symmetry; and autoregressive moving average. The Akaiki Information Criterion (AIC) was used to compare competing models and a reduction of >2 was deemed to be an improvement in model performance. Confidence intervals were derived from an ordinary non-parametric bootstrap procedure because this conservative method makes no *a priori* assumptions about the distribution of the data (Carpenter and Bithell, 2000). Where growth was not observed, a linear mixed effects model was fitted to the data incorporating the temporal autocorrelation structures described above. For the linear models, goodness of fit was quantified by calculating marginal and conditional R^2 values, as described by Nakagawa and Schielzeth (2013).

Where *E. coli* concentration growth was observed, the day of maximum *E. coli* concentration was determined for each replicate individually. A $\log_{10}$ transformation was required to normalise the data after which a one-way ANOVA and a Tukey test was applied to investigate whether the day of maximum *E. coli* concentration differed significantly between livestock/temperature treatments.

Moisture content of faeces was measured as a percentage and was thus bounded. Therefore, a logit transformation was applied and an Anderson – Darling normality test used to confirm the transformed data were from a normal distribution. A two-way ANOVA and a Tukey test was applied to determine any differences in the moisture content of faeces from the three livestock types collected during different seasons.

Data processing and analysis was implemented in the R statistics package utilising a number of third party plugins (R Core Team, 2015; Wickham and Francois, 2016; Graves et al., 2015; Pinheiro et al., 2015; Ogle, 2015; Neuwirth, 2014).

3. Results and discussion

The high resolution monitoring in this study has, for the first time, provided data that have enabled the development of a non-linear, asymptotic description of *E. coli* proliferation in livestock faeces immediately following deposition. Results are based on a total of 364, 383 and 255 faecal samples taken from beef cattle, dairy cattle and sheep faeces, respectively. This represents the

most sustained period of high frequency sampling of three livestock faecal types thus far reported, providing an unparalleled evidence base with which to characterise and better understand *E. coli* growth patterns in livestock faeces. In particular, the study highlights how existing catchment scale modelling approaches, which often assume a simple linear decay function, are unlikely to capture the complexity of *E. coli* persistence in fresh faeces (Cho et al., 2016).

Initial concentrations of *E. coli* in livestock faeces are shown in Table 2 and, with the exception of the spring beef treatment, were in line with previously published research for all livestock and season combinations; the concentration of *E. coli* in the spring beef experiment were approximately 0.5 $\log_{10}$ CFU g^{-1} of dry faeces lower than values commonly reported in the literature (e.g. Hodgson et al., 2009; Muirhead and Littlejohn, 2009; Oliver et al., 2010; Oladeinde et al., 2014). Both temperature regimes applied to dairy faeces for the summer treatment resulted in maximum concentrations of *E. coli* that were greater than maximum concentrations reported in previous published studies (Table 2). The summer and summer +2 °C treatments exceeded the previously reported maximum by 0.32 and 0.49 $\log_{10}$ CFU *E.coli* g^{-1} dry faeces, respectively (Muirhead et al., 2005; Soupir et al., 2008; Oladeinde et al., 2014; Oliver et al., 2010; Van Kessel et al., 2007). While efforts were made to simulate field conditions these inflated maxima may be due to the experiment being carried out inside a CEF where faecal deposits were isolated from stressors present in the field, and did not encounter, for example, cell wash-out following rainfall. Furthermore, under field conditions soil macrofauna such as beetles and earthworms break up faeces, which can affect the survival of *E. coli* (Ryan et al., 2011; Pedersen and Hendriksen, 1993). Given that the faecal material in this experiment may have been protected from some factors experienced in the field, extrapolation of our regrowth model to field conditions must be done with a degree of caution, with recognition that the experiment was undertaken to develop greater insight into what drives patterns of *E. coli* regrowth.

When *E. coli* growth was observed it did not differ significantly between temperature/stock type combinations ($p \geq 0.05$). Day 13 (sd = 6) was, on average, the timing of maximum *E. coli* concentration, which was similar to previous studies with an average of 9 (sd = 9) days (Muirhead et al., 2005; Soupir et al., 2008; van Kessel et al., 2007; Oladeinde et al., 2014; Oliver et al., 2010). An earlier day of maximum concentration was observed for beef cattle faeces in the spring treatment; little or no *E. coli* growth was associated with these faeces and early maximum *E. coli* concentrations only arose due to a small deviation from a static phase of *E. coli* persistence. These data suggest that livestock type and temperature do not affect the time taken to reach a maximum *E. coli* concentration during regrowth; this is important in that it might present an opportunity to simplify the parameterisation of *E. coli* persistence in modelling efforts. However, our experiment was conducted under moderate temperatures typical of spring and summer in the UK. Regions of the world where temperatures are higher, and closer to the optimum for *E. coli* replication (37 °C), may promote further *E. coli* regrowth (Oliver and Page, 2016).

An asymptotic model form provided good fit to the data for all instances of *E. coli* regrowth. Model results are shown in Fig. 1 and asymptotic model parameters for the models associated with each of the livestock types are given in table 3. The asymptotic form contains three parameters: a starting value, exponential rate constant and an asymptote. A fixed effect of temperature category was applied to the asymptote parameter only because: (i) the observed data showed no significant difference in the time taken to reach maximum concentration between temperature treatments, and (ii) the starting value was expected to be similar because, for each model, the source of faeces is the same. For the data associated with beef cattle and sheep faeces, a solution for an asymptotic model was not achieved when the data from the spring temperature treatment was included (i.e. no growth was observed for those treatments). A solution was achieved when the summer data was considered separately. For these models, including an effect of temperature sensitivity (present/present +2 °C) on the asymptote did not improve model performance (i.e. the reduction in AIC was <2). For the dairy faeces data, an asymptotic model was fitted to both the spring and summer data with the inclusion of the temperature sensitivity effect on the asymptote improving model performance (AIC reduced by 11.98). A plot of the autocorrelation function associated with the models for the beef and dairy cattle experiment showed some temporal autocorrelation between residuals at different time points. The best performing autocorrelation structures were a compound-symmetry (AIC reduced by 12.87) and auto-regressive moving average autocorrelation structure (one auto-regressive parameter and one moving average parameter) (AIC reduced by 12.30) for the beef and dairy cattle treatments, respectively.

No *E. coli* growth was observed during the spring treatments for beef cattle and sheep faeces; therefore, linear mixed effects models were fitted to these data. The *E. coli* concentrations in beef faeces decreased at a rate of −0.04 (−0.04, −0.03) $\log_{10}$ CFU g^{-1} dry faeces per day and had an intercept of 4.78 (4.19, 5.30) $\log_{10}$ CFU g^{-1} dry faeces (numbers in parentheses show lower and upper 95% confidence intervals). Marginal and conditional R^2 values associated with this model were 0.13 and 0.76, respectively. For the sheep data, a linear model showed a negative relationship between

Table 2
Average (n = 5) initial, maximum, and day of maximum *E. coli* concentration for faeces from three different livestock types under four temperature regimes. All values are given as $\log_{10}$ CFU/g dry faeces.

Livestock	Temperature	Mean initial *E. coli* concentration ($\log_{10}$ CFU g^{-1} dry wt. faeces)	Mean maximum *E. coli* concentration ($\log_{10}$ CFU g^{-1} dry wt. faeces)	Mean day of maximum *E. coli* concentration	Mean initial moisture content (%)
Beef	Spring	3.87	5.02	6.28	87.22
	Spring +2 °C	5.22	5.96	1.90	85.46
	Summer	6.03	8.51	15.76	91.22
	Summer +2 °C	5.70	8.65	13.16	90.39
Dairy	Spring	6.19	8.11	20.42	84.90
	Spring +2 °C	6.18	8.28	11.83	85.01
	Summer	6.60	8.87	10.72	86.54
	Summer +2 °C	6.58	9.10	10.52	85.62
Sheep	Spring	6.79	8.20	11.52	66.88
	Spring +2 °C	6.41	8.59	16.89	67.34
	Summer	7.03	8.73	10.36	71.85
	Summer +2 °C	7.18	8.88	13.96	71.98

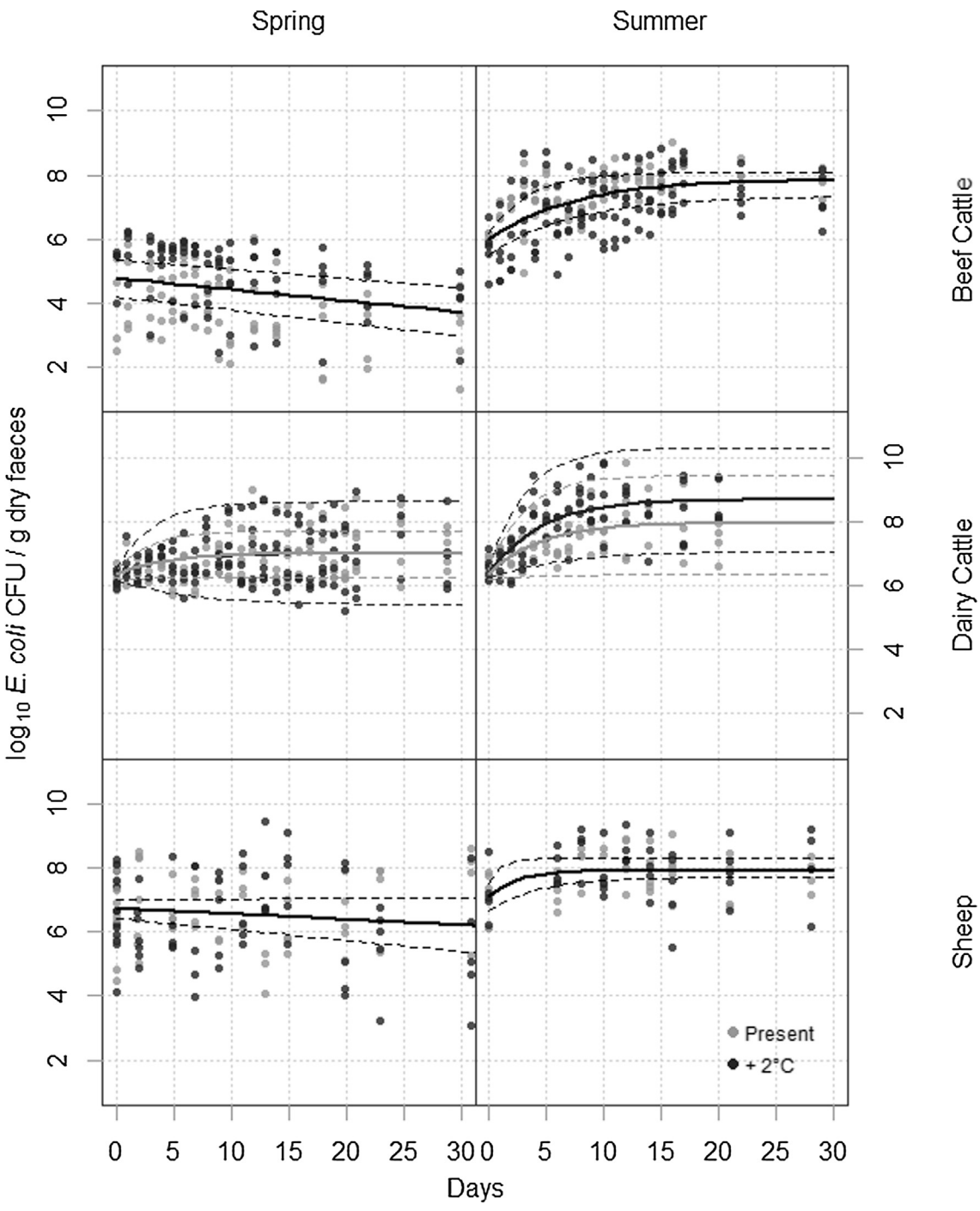

Fig. 1. Scatter plot of *E. coli* concentrations through time. Where growth occurred lines are predictions of nonlinear (asymptotic exponential) mixed effects modelling. Where no growth was apparent lines illustrate linear mixed effects models. Dashed lines indicate 95% confidence intervals derived from normal non parametric bootstrap. Where the prediction is coloured black there was no improvement in model performance (change in AIC < 2) when the present average/present average +2 °C treatment was incorporated.

E. coli concentration and days since defecation (slope parameter = −0.02 (−0.03, 0.00) $\log_{10}$ CFU g^{-1} per day, intercept = 6.72 (6.41, 7.01) $\log_{10}$ CFU g^{-1}). However, this was associated with a large uncertainty and a gradient of 0 within the 95% confidence interval. Marginal and conditional R^2 values were 0.02 and 0.06 respectively, suggesting that the model does not explain the variance in the data (Fig. 1). This weak relationship may be due to *E. coli* concentrations remaining largely static within sheep faeces under the spring temperature regime, with variation between individual deposits greater than the change over 30 days.

Where *E. coli* growth was observed, separate non-linear, asymptotic models were fitted to the different livestock types because a model could not be fitted when all instances of growth across all livestock type/season combinations were included. Separate models were justified given the marked differences in the management of beef cattle, dairy cattle and sheep. For example there will be differences in the diet and grazing regimes of different livestock, which can impact on *E. coli* shedding (Oliver, 2014). In the future, it may be possible to develop a unified model of *E. coli* persistence in livestock faeces but this would require a large amount of

Table 3
Table of model parameters associated with asymptotic models for each of the livestock types.

		Intercept $\log_{10}$ CFUg^{-1}	Exponential rate constant c day^{-1}	Asymptote a $\log_{10}$ CFUg^{-1}	Magnitude of growth b (Asymptote - Intercept) $\log_{10}$ CFUg^{-1}
Beef	Spring	–	–	–	–
	Spring +2 °C		–	–	–
	Summer	−5.88 (5.51, 6.20)	−0.183 (−0.134, −0.275)	7.72 (7.35, 8.09)	1.84
	Summer +2 °C				
Dairy	Spring			7.02 (5.41, 8.62)	0.67
	Spring +2 °C	6.35 (6.19, 6.49)	−0.230 (−0.170, −0.323)	7.00 (6.26,7.71)	0.65
	Summer			7.95 (6.35,9.43)	1.6
	Summer +2 °C			8.68 (7.05,10.29)	2.33
Sheep	Spring	–	–	–	–
	Spring +2 °C	–	–	–	–
	Summer	7.10 (6.63, 7.52)	−0.411 (−0.235, −0.9139)	7.91 (7.70, 8.28)	0.81
	Summer +2 °C				

supplementary information on different management regimes, which at present is unavailable.

Parameters derived from the models can be used to compare and contrast *E. coli* persistence in the faeces of the different livestock studied. For example, the magnitude of growth can be taken as the asymptote minus the intercept. For dairy cattle faeces, the summer +2 °C temperature treatment showed the highest level of *E. coli* growth, whilst the dairy cattle faeces under the spring temperature treatments showed the lowest increase in *E. coli* concentrations. Model results highlighted that only dairy cattle faeces under the summer temperature regime showed a difference in the magnitude of growth between the present and +2 °C treatment, with the +2 °C treatment showing an increase of 0.73 $\log_{10}$ CFUg^{-1} dry faeces more *E. coli* growth relative to the standard summer temperature treatment. For beef cattle faeces, *E. coli* concentration growth was only observed in the summer temperature treatments with only a small difference in *E. coli* growth between the summer and +2 °C temperature treatments. The magnitude of *E. coli* growth observed in the beef cattle faeces within the summer temperature treatments was comparable to that recorded in dairy cattle faeces. *E. coli* growth was only observed in sheep faeces under the summer temperature treatment with no difference in the magnitude of *E. coli* growth between summer and +2 °C temperature treatments. For all livestock types, sheep faeces showed the lowest *E. coli* growth during the summer experiment. The rate constant of the asymptotic equation provided insight into the rate of *E. coli* growth. In order of fastest to slowest for rates of *E. coli* growth: sheep faeces > dairy cattle faeces > beef cattle faeces (Table 3).

Increased potential for *E. coli* growth during warmer temperatures for all three livestock types was observed. Moisture content (Table 2) also appeared to affect the rate of *E. coli* concentration change. The effect of moisture on the change in *E. coli* concentration through time was observed by taking the moisture content associated with an individual sample and the rate of *E. coli* concentration change between that observation and the corresponding previous observation. *E. coli* growth in dairy and beef cattle faeces was more likely to be observed at higher moisture contents with beef cattle faeces showing some *E. coli* growth at lower moisture contents. However, no clear pattern was evident for sheep faeces and growth rates appeared to decrease as deposits dried over time. A two-way ANOVA revealed that beef cattle faeces collected during the spring were 4.5% drier than those collected in the summer (Fig. 2) and this reduction in moisture content associated with spring faeces may have influenced differences in the survival of *E. coli* in fresh beef cattle faeces that were observed between the spring and summer temperature treatments (Oliver and Page, 2016). Reductions in the moisture content of faeces are likely due to differences in the diet of beef cattle in the two seasons studied. For example, the diet of beef cattle in spring was more likely to be supplemented with hay, silage and concentrates, whereas in the summer the diet was dominated by fresh grass. The effect of diet on *E. coli* persistence in faecal deposits is likely to be multifaceted

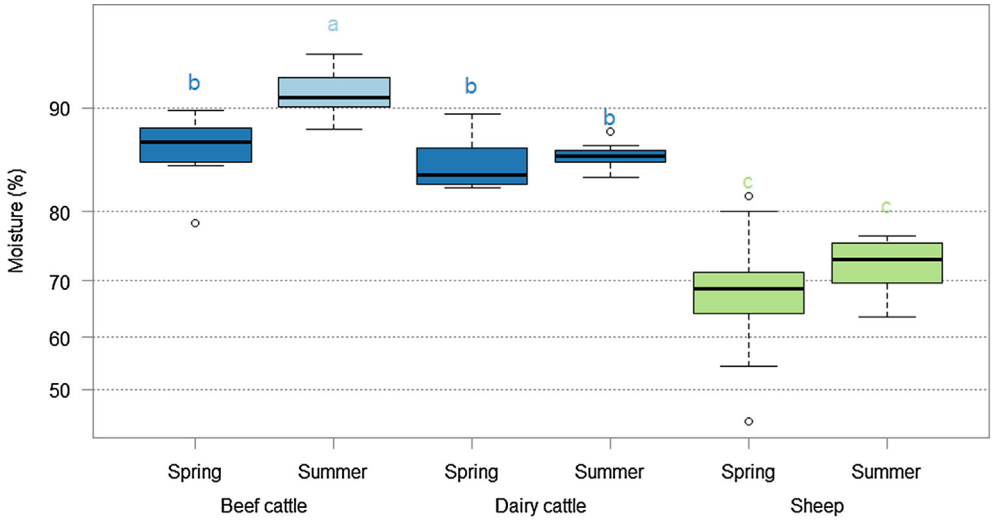

Fig. 2. Boxplot showing logit transformed initial moisture content in the faeces of three livestock types from two seasons. Different letters and colours illustrate where results of a Tukey post-hoc test revealed differences between livestock and season combinations. Y axis labels have been back transformed to improve interpretability.

with moisture being one of many controlling factors; for example, Donnison et al. (2008) reported a reduced burden of FIOs in cattle that were fed silage compared to cattle grazing pasture and suggested reductions in rumen pH as a controlling variable. If the difference in *E. coli* concentrations in beef cattle faeces between seasons is extrapolated to a catchment scale the differences in the size of the landscape reservoir of *E. coli* through time are likely to be marked, reinforcing the importance of accounting for seasonal persistence profiles of *E. coli* in catchment-scale models (Wang et al., 2018a). Therefore, further investigation into the influence of cattle diet on microbial concentrations in faeces is warranted (Berry et al., 2017).

A relationship between moisture content and *E. coli* growth rate was not apparent in sheep faeces, which were significantly drier ($p < 0.05$) than the faeces of the beef and dairy cattle. This reduced moisture content may have contributed to the lack of *E. coli* growth in sheep faeces exposed to the spring temperature treatments and the very limited growth relative to dairy and beef cattle observed in the summer temperature treatments. For sheep faeces in the summer temperature treatments there were a few observations with high moisture content. These observations occurred during the first five days of the experiment with rapid drying occurring over subsequent days. Despite higher moisture content, *E. coli* growth was not apparent suggesting that moisture content is not the only limiting factor for *E. coli* growth in sheep faeces. However, variations in the survival of *E. coli* in the faecal reservoir due to changes in moisture content and temperature, and their interactive effects, are likely to contribute to observed seasonal variations of watercourse FIO pollution (e.g. such as that observed in Muirhead and Meenken, 2018).

This study, operating within a CEF, succeeded in replicating *E. coli* regrowth in livestock faeces, which has previously been observed under field conditions (Oladeinde et al., 2014). A key difference in our study relative to other laboratory studies is that temperature was not statically held at a single value; it varied diurnally following a sinusoidal wave form. This would suggest that diurnal variation in temperature can somehow promote a mechanism to drive *E. coli* regrowth, which controlled experiments under a constant temperature cannot replicate. From our experiment it is unclear whether it is the size of diurnal variation or the absolute temperature that drives protracted *E. coli* survival because there was only a small difference (7.89 vs 8.14 °C) in the diurnal variation between the seasons studied. It is worth noting that the CEF provided a diurnal cycle with a consistent cycle of temperature and UV irradiation maxima and minima, thus everyday had the same pattern of variation. In the natural environment these cycles would exhibit more day-to-day variability.

Little is known about the mechanisms that promote *E. coli* regrowth in faeces but it is possible morphological changes in *E. coli* cells may promote more rapid growth of *E. coli* under varying temperature compared to static temperature regimes. Jones et al. (2004) observed *E. coli* growth when refrigerated *E. coli* in nutrient broth was exposed to warmer temperatures at a cycle of 12 h and suggested the formation of filamentous *E. coli* at temperatures colder than the minimum for growth as a driver. Likewise, Mattick et al. (2003) showed how refrigerated filamentous *Salmonella* spp. rapidly multiply when temperature was increased. Thus, it is possible that the development of filamentous forms of *E. coli* and subsequent rapid division over a diurnal temperature variation contributes to protracted *E. coli* survival in livestock faeces under variable field conditions compared to static temperature conditions. Further investigation into the influence of *E. coli* morphology on nuances in its survival in livestock faeces is therefore warranted.

Replicating *E. coli* regrowth, as seen under field conditions, in a laboratory setting demonstrates the potential for improvements in reductionist, laboratory-based studies. For example, embedding a more accurate (but controlled) representation of environmental drivers into mechanistic studies via more sophisticated CEF functionality can reveal new insight that would be overlooked by simplistic constant temperature regimes (Wang et al., 2018b). Clearly, interactions between multiple environmental variables in the field make it difficult to identify variables that control profiles of *E. coli* persistence; however, our study demonstrates that CEFs can be used to control some environmental variables while varying others for a more detailed investigation relative to static-temperature laboratory studies.

Diffuse pollution mitigation measures are costly and occupy valuable productive land, and therefore measures must be targeted toward areas where they will contribute to the greatest improvement in water quality and the least disruption to catchment stakeholders (Lloyd et al., 2019). Ultimately, the results from our study can be used to improve understanding of the relative contribution of different livestock types to microbial watercourse pollution. Field burden models have shown how total *E. coli* reaches an asymptote as introduction of new *E. coli* via fresh faecal deposits equilibrates with die-off of *E. coli* in existing faecal deposits (Oliver et al., 2010). Therefore, the peak concentration of *E. coli* within individual deposits is one way to characterise the hazard from faecal deposits and can be calculated by multiplying the asymptote of the models and the dry weight of the deposits. For deposits under the spring temperature treatment, dairy cattle contributed the most *E. coli* per deposit (9.35 $\log_{10}$ CFU) followed by sheep (7.80 $\log_{10}$ CFU) with beef cattle contributing the least *E. coli* per deposit (7.06 $\log_{10}$ CFU). For the summer experiment the peak *E. coli* hazard followed the order of dairy cattle (10.29 $\log_{10}$ CFU) > beef cattle (10.00 $\log_{10}$ CFU) > sheep (8.98 $\log_{10}$ CFU). Of course, defecation rates and variable stocking densities would also influence the level of hazard associated with faecal loading of pasture and must also be accounted for when making landscape scale predictions of *E. coli* burden. While this provides a useful concept, national scale inventories of faecal deposit mass by livestock age, faecal deposit *E. coli* concentrations and defecation rates are needed to supplement the data collected here. This would help to make predictions of the relative hazard associated with different livestock faeces more robust. Such characterisation of microbial hazards can, in turn, be integrated into existing risk based models of diffuse pollution transfer, for example SCIMAP (Porter et al., 2017). Risk based approaches may be especially useful in the study of catchment microbial dynamics because of the relative lack of understanding on the fate and transfer of FIOs in the landscape compared to other agricultural diffuse pollutants (Oliver et al., 2016).

4. Conclusion

FIO survival at the landscape level is a key controlling factor on the extent to which river networks become contaminated following rainfall and is a key component of catchment scale predictions of FIO contamination. A linear approach to modelling FIO survival is likely to underestimate the burden of *E. coli* in fresh livestock deposits because it does not account for *E. coli* proliferation, under favourable conditions, as observed in field studies, and now also captured within a CEF mimicking fluctuating environmental conditions. The model developed as part of the current study provides a critical preliminary step towards a framework of accounting for seasonal variations in *E. coli* growth associated with livestock faeces at the catchment scale. However, management practices (for example diet and livestock housing), which vary throughout the year and between farms, are also likely to influence *E. coli* survival. The interaction of agricultural management practices and

meteorological variables presents a contemporary challenge for the field of catchment microbial dynamics and further understanding is needed if the risks to ecosystem services related to clean and safe water are to be fully understood and predicted. While it is relatively simple to apply growth models to existing *E. coli* data it will be much more challenging to code *E. coli* growth functions into environmental models for extrapolation and forecasting. However, the analysis presented here can inform future development of microbial pollution risk assessment and decision support tools. This is important to ensure that catchment scale predictions of *E. coli* accumulation and persistence on land are robust, accurate and evidence-based, and thus more useful to the policy and decision-making community.

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